

STATE OF MONTANA  
DEPARTMENT OF AGRICULTURE

A Laboratory Study of the Breakdown of 1080 Residues  
in Frozen and Incubated Ground Squirrel Carcasses

In 1979 and 1981 Montana was granted specific exemptions, under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act, to use compound 1080 to control Columbian ground squirrels. In granting these exemptions, EPA requested that the Montana Department of Agriculture initiate studies on the non-target mortalities and potential population reductions to non-target animals caused by 1080 poisoning. Consequently, a cooperative effort was undertaken with the Montana Cooperative Wildlife Unit at the University of Montana to address this issue.

One part of the issue of non-target mortality involves that of secondary poisoning or the hazard to animals such as coyotes and eagles that scavenge on carcasses of 1080-killed animals. In addressing this matter it was felt that more information was needed on the behavior of 1080 residues in poisoned ground squirrel carcasses. To determine the true hazard of secondary poisoning a study of the breakdown of 1080 in carcasses was necessary.

Observations in 1979 indicated that 1080 might be breaking down rapidly in ground squirrel carcasses. The Montana Cooperative Wildlife Unit fed 1080 killed ground squirrels to penned coyotes in 1979. Coyotes that had been captive for some time were accustomed to accepting food from humans and immediately consumed freshly killed squirrels. These coyotes died within several days of consumption of 1080 killed squirrels. Upon necropsy, they appeared to have died from cardiac failure and had extensive hemorrhages in the spleen and liver. One coyote, in captivity a short time, did not readily accept food from humans. This coyote allowed ground squirrels to lay in the pen for a day or longer before consuming them. The coyote consumed eight 1080 killed ground squirrels and lived for 20 days. At death, this coyote appeared to have died from bacterial infections of the spleen and liver. Thus, sub-lethal doses of 1080 may have resulted in secondary infections. One explanation of these observations was that 1080 residues were breaking down in the ground squirrel carcasses.

In 1980, a laboratory study was conducted to investigate the breakdown of 1080 residues in Columbian ground squirrel carcasses. This report summarizes the findings of that study.

METHODS

Columbian ground squirrels (Spermophilis columbianus) were live trapped in Missoula County, Montana, in March 1981. Because of the activity pattern of Columbian ground squirrels, only males could be captured at this time of year. Squirrels were transported to Bozeman, Montana and housed in 12X9X12 inch metal cages. All animals were acclimated to laboratory conditions for 7 days prior to testing and fed a standard lab diet consisting of Purina Formulab Chow #5008 and water ad lib. Prior to testing, animals were fed only rolled oats for 3 days.

Although no specific standard methods have been proposed for this type of study ASTM standards were consulted and modified, particularly those of Palmateer (1979).

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Analysis of 1080 residues was conducted using techniques described in Ray and Associates et al. (1981) however these techniques were modified to include analysis by gas chromatograph/mass spectrophotometer to increase sensitivity of detection.

To further refine analytical techniques an evaluation of 1080 breakdown in frozen tissues was undertaken. Carcasses are often frozen by field investigators for later analysis at the convenience of the lab with the assumption that breakdown is halted by freezing. This assumption was questioned. Analyses of 1080 residues in frozen ground squirrel tissue were conducted to determine if breakdown of 1080 does occur (Holding Study).

#### Residues in Frozen Carcasses (Holding Study)

Each of 5 ground squirrels was given 0.16<sup>1</sup> ounces of oat groats containing 0.05% compound 1080. The amount of this bait that was consumed was measured. Of these squirrels one died after 5 hours. The others, although still living, were comatose and in an irreversible condition characteristic of 1080 poisoning (Atzert 1971). These animals were sacrificed after 18 hours. Stomach and contents were removed from all squirrels and were combined and blended to form one composite sample. The composite sample was divided into six subsamples and 4 of these frozen. The two unfrozen samples were analyzed immediately for 1080 residues and frozen subsamples were analyzed 5 and 14 days after freezing, two subsamples at each interval.

#### Residues in Incubated Carcasses

For administering by gavage, a solution of 1080 in distilled water was prepared. Each test squirrel was anesthetized with metofane (methoxyflurane) and administered 2.84<sup>2</sup> mg 1080 (90% pure technical product) in 3 ml. distilled water using a number 18 animal feeding needle. Solutions were introduced directly into each animal's stomach. A control group was treated similarly using a clean syringe and feeding needles to administer 3 ml. of untreated distilled water.

After death, 4 squirrels, 3 test and 1 control, were analyzed immediately for 1080 residues. Other carcasses in groups of 4 were incubated at 24-27°C for periods of 48, 72, and 150 hours and then analyzed. Control carcasses were also incubated for each period.

For each squirrel the stomach and its contents and 20-30 grams of muscle tissue were analyzed separately.

A summary of incubation times, treatments, and weights for individual animals is given in Table 1.

### RESULTS

A summary of 1080 residues from the incubation study is given in Table 2. Residues are reported in parts per million and percent recovery based on a spiked

- 1-The amount specified on the 1080 label to be applied per burrow to control Columbian ground squirrels.
- 2-The amount of 1080 present in 0.16 ounces of 1080-treated oats used for Columbian ground squirrel control.





sample is given. Incubation times are also presented. Due to varying hours to death for individual ground squirrels and, because of lab schedules, incubation times are close approximates of the anticipated 48, 72, and 150 hours. Despite variation among squirrel responses to 1080 and among samples indications were that 1080 residues decreased with increasing incubation times.

Percent recovery of 1080 residues based on a spiked sample varied among analyses. Tables 3 through 7 show 1080 concentrations recalculated to 100%. This information is useful for the elimination of analytical error due to poor spike recovery.

Results from the holding study are given in Tables 8 and 9. Results indicate that 1080 residues decrease with increasing storage (freezing) time. Total residues after 14 days of storage decreased 79% for muscle and 49% for stomach and content compared to 0-hour residues (Figure 1).

### DISCUSSION

Interpretation of this study was difficult due to unpredicted variation in the data. Residues varied among individual animals even though dosage and holding times were the same. Sources of variation are due, in part, to the different responses of ground squirrels to 1080 as shown, for example, by variable times to death (Table 1). Future investigations should account for variables that might influence residue levels including physiological health, weight, age, sex, and stomach content of test animals. There is also some question as to the accuracy of the analytical procedure. We have no explanation for the large variation in spike recovery, for example, 100% recovery of stomach and contents at "0 hours" and only 5% recovery at "150 hours".

All tests did indicate, however, that 1080 residues decrease over time in incubated and frozen animal carcasses.

The holding (freezing) study did eliminate variables due to individual responses to 1080. For this reason, data from this experiment may be more reliable. The indication is that 1080 continues to break down even in frozen samples. This points out a need for rapid analysis of any samples for 1080 residues.

The incubation study indicates a breakdown of 1080 residues at conditions approximating daytime temperatures. To define a rate of decomposition further lab studies are necessary and should be supported by field studies. It is recommended that studies be continued, possibly as a cooperative federal and state effort.

### LITERATURE CITED

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TABLE 1  
LAB TREATMENTS AND WEIGHTS OF COLUMBIAN GROUND  
SQUIRRELS ANALYZED FOR 1080 RESIDUES

(Freezing Study)

<u>Animal Number</u>	<u>Weight (gms)</u>	<u>Treatment (1080)</u>	<u>Post Treatment Hrs. to Death</u>	<u>Preparation</u>
3	380	.61 gm. grain (.05% 1080)	5	6:00 a.m., 4-8. Cold Storage 7 hrs.
4	392	1.22 gm grain (.05% 1080)	18*	11:00 a.m., 4-8. when scarified.
5	304	1.70 gm grain (.05% 1080)	18*	11:00 a.m., 4-8. when scarified.
6	314	2.65 gm grain (.05% 1080)	19*	12:00 a.m., 4-8. when scarified.
7	484	2.8 gm grain (.05% 1080)	19*	12: a.m. 4-8. when scarified.

\*Animal Sacrificed

(0-Hour Incubation -- Gavage at 12 p.m., 4-13-81)

10	466	2.8 mg gavage	23	1:30 p.m., 4-14 stored a rm. temp. 2.5
11	476	2.8 mg "	9	6:30 a.m. 4-14 cold storage 9 hrs.
12	410	2.8 mg "	44	Carcass incinerated.
13	444	2.8 mg "	38	6:15 a.m., 4-15 Rm. Temp. 5 hrs.
14	—	control	—	—

(48-Hour Incubation -- Gavage at 3:00 p.m. 5-1-81)

21	424	2.8 mg Gavage	4	55 hrs. at 24°C 4:30 a.m., 5-4
23	570	2.8 mg "	4	55 hrs. at 24°C 4:30 a.m., 5-4
24	568	2.8 mg "	4	56 hrs. at 24°C 5:00 a.m., 5-4
27	538	control	—	42 hrs. at 24°C 5:30 a.m., 5-4

(72-Hour Incubation -- Gavage at 8 a.m., 4-15-81)

15	430	2.8 mg Gavage	7	110 hrs. at 27°C 5:30 a.m., 4-20
16	440	2.8 mg "	28	88 hrs. at 27°C 5:30 a.m., 4-20
17	370	2.8 mg "	10	107 hrs. at 27°C 6:30 a.m., 4-20
18	—	control	—	89 hrs. at 27°C 6:30 a.m., 4-20



TABLE 1 (Continued)

(150-Hour Incubation -- Gavage at 9:00 a.m. 5-4-81)

<u>Animal Number</u>	<u>Weight (gms)</u>	<u>Treatment (1080)</u>	<u>Post Treatment Hrs. to Death</u>	<u>Preparation</u>
29	450	2.8 mg. Gavage	31	156 hrs. at 27 <sup>0</sup> C. 4:00 a.m., 5-12
32	530	2.8 mg "	34	152 hrs. at 27 <sup>0</sup> C. 4:30 a.m., 5-12
33	470	2.8 mg "		153 hrs. at 27 <sup>0</sup> C. 5:00 a.m., 5-12
34	590	control	—	157 hrs. at 27 <sup>0</sup> C. 5:15 a.m., 5-12



TABLE 2  
SUMMARY OF 1080 RESIDUES REPORTED IN PARTS PER MILLION (ppm) FOR  
GROUND SQUIRRELS GIVEN 1080 VIA GAVAGE

	<u>"0 Hour"</u>		<u>Animal Number</u>	<u>"48 Hours"(5)</u>	
	<u>Animal Number</u>	<u>ppm 1080</u>		<u>ppm 1080</u>	<u>Hours Incubated</u>
Muscle 1	13	2.1 (2)	21	1.6	55
Muscle 2	10	1.5 (3)	23	0.38	55
Muscle 3	11	1.5 (4)	24	1.4	55
Average		1.7		1.13	55
Muscle "Blank"	14	none detected	27	---	--
Muscle "Spike" (% recovery)		-----		45%	42
Stomach & Contents 1	13	1.5 (1)	21	2.16	55
Stomach & Contents 2	10	19.3 (3)	23	17.9	55
Stomach & Contents 3	11	25.9 (4)	24	8.3	55
Stomach & Contents "Blank"	14	none detected	27	---	--
Stomach & Contents "Spike" (% recovery)		100% (6)		25%	42
	<u>"72 Hours"</u>		<u>Animal Number</u>	<u>"150 Hours"(5)</u>	
	<u>Animal Number</u>	<u>ppm 1080</u>		<u>ppm 1080</u>	<u>Hours Incubated</u>
Muscle 1	15	0.41	33	0.39	153
Muscle 2	16	0.63	32	0.36	152
Muscle 3	17	0.49	29	0.27	156
Average		0.51		0.34	154
Muscle "Blank"	18	---	34	---	---
Muscle "Spike" (% recovery)		62%		30%	157
Stomach & Contents 1	15	2.16	33	0.90	153
Stomach & Contents 2	16	1.70	32	0.54	152
Stomach & Contents 3	27	2.03	29	0.41	156
Stomach & Contents "Blank"	18	---	34	---	---
Stomach & Contents "Spike" (% recovery)		16%		5%	157





TABLE 2 (Continued)

- NOTES:
- (1) Little food material in stomach
  - (2) Died overnight, then remained at room temperature for approximately 5 hours. Prepared 6:15 a.m. and analysis was started 8:00 a.m., 4/15/81.
  - (3) Died at 11:00 a.m. and analysis started 3:00 p.m. on 4/14/81.
  - (4) Died at 9:00 p.m., 4/13/81 and analysis started 9:30 a.m. on 4/14/81.
  - (5) New 1080 solution was used.
  - (6) Separate study (CR #2838 and 2839)
  - (7) Animal number corresponding to that on Table 1 is given in parentheses for each analysis.



TABLE 3  
RESULTS OF TABLE 2 RECALCULATED TO 100% RECOVERY

	ANIMAL NUMBER	"0 HOUR"(1)	ANIMAL NUMBER	"48 HOURS"	ANIMAL NUMBER	"72 HOURS"	ANIMAL NUMBER	"150 HOURS"
Muscle 1	13	4.2 ppm	21	3.6 ppm	15	0.66 ppm	33	1.26 ppm
Muscle 2	10	3.0 ppm	23	0.84 ppm	16	1.02 ppm	32	1.16 ppm
Muscle 3	11	3.0 ppm	24	3.1 ppm	17	0.79 ppm	29	0.87 ppm
Stomach & Contents 1	13	1.5 ppm	21	8.6 ppm	15	13.5 ppm	33	18.9 ppm
Stomach & Contents 2	10	19.3 ppm	23	72.0 ppm	16	10.6 ppm	32	10.8 ppm
Stomach & Contents 3	11	25.9 ppm	24	33.0 ppm	17	12.6 ppm	29	8.2 ppm

NOTE: (1) For muscle: 50% recovery was used, at 0 Hour.  
For contents: 100% recovery was used at 0 Hour.  
For 48, 72 and 150 Hours, the % recoveries are indicated in Table 1.

TABLE 4  
"0 HOUR" INCUBATION RESIDUES CALCULATED TO 100% RECOVERY

	Sample Weight	4g 1080 Introduced into Animal	4g 1080 Remained as Reported	4g 1080 Remained Corrected to 100% Recovery	% 1080 Remained Corrected to 100% Recovery
13 Stomach & Contents 1	6.7 g	2800	10	10	0.36
10 Stomach & Contents 2	17.0 g	2800	328	328	11.7
11 Stomach & Contents 3	15.1 g	2800	391	391	14.0

NOTES: 100% Spike Recovery was used for calculations.





TABLE 5  
"48 HOUR" INCUBATION RESIDUES CALCULATED TO 100% RECOVERY

Sample Weight	4g 1080 Introduced into Animal	4g 1080 Remained as Reported	4g 1080 Remained Corrected to 100% Recovery	%1080 Remained as Reported	%1080 Remained Corrected to 100% Recovery
21 Stomach & Contents 1 5.6 g	2800	12	48	0.43	1.7
23 Stomach & Contents 2 9.7 g	2800	173	698	6.2	24.9
24 Stomach & Contents 3 7.7 g	2800	63	255	2.3	9.0

NOTES: 25% Spike Recovery was used for calculations.

TABLE 6  
"72 HOUR" INCUBATION RESIDUES CALCULATED TO 100% RECOVERY

Sample Weight	4g 1080 Introduced into Animal	4g 1080 Remained as Reported	4g 1080 Remained Corrected to 100% Recovery	%1080 Remained as Reported	% 1080 Remained Corrected to 100% Recovery
15 Stomach & Contents 1 15.0 g	2800	32	202	1.1	7.2
16 Stomach & Contents 2 10.8 g	2800	18	115	0.64	4.1
17 Stomach & Contents 3 14.0 g	2800	28	175	1.0	6.3

NOTES: 16% Spike Recovery was used for calculations.

TABLE 7  
"150 HOUR" INCUBATION RESIDUES CALCULATED TO 100% RECOVERY

Sample Weight	4g 1080 Introduced into Animal	4g 1080 Remained as Reported	4g, 1080 Remained Corrected to 100% Recovery	%1080 Remained as Reported	% 1080 Remained Corrected to 100% Recovery
33 Stomach & Contents 1 10.1 g	2800	9	180	0.32	6.4
32 Stomach & Contents 2 14.8 g	2800	8	160	0.29	5.7
29 Stomach & Contents 3 16.4 g	2800	7	134	0.25	4.9

NOTES: 5% Spike Recovery was used for calculations.



TABLE 8  
RESIDUES FROM FROZEN STOMACH AND CONTENT OF 1080 KILLED GROUND SQUIRRELS

<u>Analysis Started</u>	<u>Time Frozen</u>	<u>Sub Sample A</u>	<u>Sub Sample B</u>	<u>Average</u>
1:00 pm, 4/8/81	0 days	25.2 ppm	26.5 ppm	25.9 ppm
7:00 am, 4/13/81	5 days	15.8 ppm	18.9 ppm	17.4 ppm
3:00 pm, 4/22/81	14 days	14.7 ppm	11.6 ppm	13.2 ppm

TABLE 9  
RESIDUES FROM FROZEN MUSCLE TISSUE OF 1080 KILLED GROUND SQUIRRELS

<u>Analysis Started</u>	<u>Time Frozen</u>	<u>Sub Sample A</u>	<u>Sub Sample B</u>	<u>Average</u>
1:00 pm, 4/8/81	0 days	0.55 ppm	0.69 ppm	0.62 ppm
7:00 am, 4/13/81	5 days	0.48 ppm	0.46 ppm	0.47 ppm
3:00 pm, 4/22/81	14 days	0.20 ppm	0.05 ppm	0.13 ppm



FIGURE 1

HOLDING TIME STUDY FOR MUSCLE  
AND STOMACH & CONTENT





